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L3: Entry 1 of 25

File: USPT

Jun 11, 2002

DOCUMENT-IDENTIFIER: US 6403303 B1

TITLE: Method and reagents for testing for mutations in the BRCA1 gene

Detailed Description Text (28):

Use of CLEAVASE (Third Wave Technologies, Inc. Madison Wis.) provides another diagnostic technique which can be used according to the invention to identify mutations in BRCA1 by determining the sizes and amounts of amplified exon fragments. CLEAVASE is an endonuclease which cuts single stranded DNA (ssDNA) molecules. Since mutant ssDNA adopts a different conformation from wild-type ssDNA, the CLEAVASE digestion products show a different array of fragments. This array of fragments can be separated and examined by electrophoresis, much like multiplex PCR fragments. The advantage of CLEAVASE is that it can detect single base substitution mutations as well as insertions and deletions. Therefore, it detects fewer false negatives than multiplex PCR, though it does not locate mutations as precisely as sequencing.

Detailed Description Text (82):

The Sequencing primer selected was a fluoresceinated version of the subservient primer (i.e. the one originally added in lesser amount). The fluorescent label allows for detection of reaction products in an automated DNA sequencer, such as the Pharmacia A.L.F.

CLAIMS:

14. A kit for testing a sample for mutations in the BRCA1 gene comprising a mixture of at least four oligonucleotide primers, said primers being selected to amplify exons 1 and 21, or a portion of each exon, of the BRCA1 gene in a multiplex amplification reaction.

15. The kit according to claim 14, wherein the primers for amplification of exons 1 and 21 are the primers identified by Sequence ID Nos. 1, 2, 69 and 70.

16. A kit for testing a sample for mutations in the BRCA1 gene comprising a mixture of at least four oligonucleotide primers, said primers being selected to amplify exons 2, 5, 9 and 14, or a portion of each exon, of the BRCA1 gene in a multiplex amplification reaction, wherein the primers used for amplification are the primers identified by Sequence ID Nos. 3, 4, 9, 10, 17, 18, 55 and 56.

17. A kit for testing a sample for mutations in the BRCA1 gene comprising a mixture of at least four oligonucleotide primers, said primers being selected to amplify exons 3, 7 and 15, or a portion of each exon, of the BRCA1 gene in a multiplex amplification reaction, wherein the primers used for amplification are the primers identified by Sequence ID Nos. 5, 6, 13, 14, 57 and 58.

18. A kit for testing a sample for mutations in the BRCA1 gene comprising a mixture of at least four oligonucleotide primers, said primers being selected to amplify exons 6, 10, 17 and 18, or a portion of each exon, of the BRCA1 gene in a multiplex amplification reaction.

19. The kit according to claim 18, wherein the primers for amplification of exons 6, 10, 17 and 18 are the primers identified by Sequence ID Nos. 11, 12, 19, 20, 61, 62, 63 and 64.

20. A kit for testing a sample for mutations in the BRCA1 gene comprising a mixture of at least four oligonucleotide primers, said primers being selected to amplify exons 4, 12 and 16, or a portion of each exon, of the BRCA1 gene in a multiplex amplification reaction.

21. The kit according to claim 20, wherein the primers for amplification of exons 4, 12 and 16 are the primers identified by Sequence ID Nos. 7, 8, 51, 52, 59 and 60.
22. A kit for testing a sample for mutations in the BRCA1 gene comprising a mixture of at least four oligonucleotide primers, said primers being selected to amplify exons 8, 13, 19 and 24, or a portion of each exon, of the BRCA1 gene in a multiplex amplification reaction.
23. The kit according to claim 22, wherein the primers for amplification of exons 8, 13, 19 and 24 are the primers identified by Sequence ID Nos. 15, 16, 53, 54, 65, 66, 75 and 76.
24. A kit for testing a sample for mutations in the BRCA1 gene comprising a mixture of at least four oligonucleotide primers, said primers being selected to amplify exons 20, 22 and 23, or a portion of each exon, of the BRCA1 gene in a multiplex amplification reaction.
25. The kit according to claim 24, wherein the primers for amplification of exons 20, 22 and 23 are the primers identified by Sequence ID Nos. 67, 68, 71, 72, 73 and 74.
26. A kit for testing a sample for mutations in the BRCA1 gene comprising a mixture of at least four oligonucleotide primers, said primers being selected to amplify exons 2 and 20, or a portion of each exon, of the BRCA1 gene in a multiplex amplification reaction, wherein the primers used for amplification are the primers identified by Sequence ID Nos. 3, 4, 65 and 66.